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CITATION:

OHSUMI, KIYOSHI. Adrenergic Innervation to The Stomach In Rat-Fluorescence Histochemical Studies. 日本外科宝函 1970, 39(4): 195-215

ISSUE DATE:

1970-10-01

URL:

http://hdl.handle.net/2433/207891

Arch. Jap. Chir. 39 (4), 195~215, Oct. 1970

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Adrenergic Innervation to The Stomach In Rat-Fluorescence Histochemical Studies

by

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Received for Publication, Sep. 5, 1970

INTRODUCTION

Using a highly specific, sensitive fluoresence histochemical technique for demonstrating catecholamines at cellular levels[6)7)8)], it has been shown that fluorescent materials accumulate evidently after ligation in the vagus nerve[38)] as well as in the splanchnic[9)] and sciatic nerves of rat[3)]. Although the findings indicate that these autonomic or somatic nerve fibers contain adrenergic components in course, no report has yet appeared regarding the physiological role played by such adrenergic components and regarding the destination where the adrenergic fibers reach.

The present experiments were performed to know where is the destination in the upper gastrointestinal tract of the fluorescent adrenergic nerve fibers mixed in the vagus nerve of rat.

MATERIALS AND METHODS

About one hundred Wistar rats weighing 250 to 300g were used. Animals were sacrificed by decapitation for excising the stomach with a part of esophagus and of duodenum. Pieces (3×3mm) were cut out from the anterior and posterior walls of the nonglandular stomach and of the body of stomach[33)], the cardia[1)48)], the antral portion[29)30)], the esophagus and the duodenum.

Some of the animals were previously subjected to surgery under pentobarbital anesthesia as follows[25)45)]: unilateral cervical vagotomy, unilateral or bilateral abdominal vagotomy, unilateral or bilateral splanchnicectomy or celiac ganglionectomy. The cervical vagotomy was performed at the level of the thyroid cartilage after exposing through a midcervical incision. The abdominal vagotomy was carried out at the middle level of abdominal esophagus after laparotomy through a midline incision. For completely cutting off the branches of the nerves[46)], the serosa and the muscularis of the esophagus were stripped off 3 mm long. The splanchnicectomy was done directly below the diaphragm after exposing through a midabdominal or a

costal arch incision. In order to completely denervate the fibers supplying the stomach[5)35)], the celiac ganglionectomy was combined with a purification of the connective tissue and nerve plexus around arteries coursing from the abdominal aorta to the left renal artery. One to fourteen days after surgical procedure the animals were sacrificed by decapitation.

Tissue pieces were subjected to histochemical studies following the method of FALCK and HILLARP[11)12)14)] with some modifications[15)16)17)]. They were quenched in isopentane cooled to −80°C with liquid nitrogen. Then the specimens were freeze-dried *in vacuo* at −30°C to −35°C for 5 to 7 days, exposed to formaldehyde gas at 80°C for 1 hour, infiltrated *in vacuo* with paraffin at 60°C for 30 minutes, and sectioned at 8μ thickness. The sections were collected on non-fluorescent slide glasses, mounted in an Entellan-xylene mixture, and analyzed and photographed in a fluorescence microscope (light source: Osram HBO 200 mercury high pressure lamp; primary filter: Schott BG 12; secondary filter: Zeiss 50). Exposure time ranged from 90 to 120 seconds on Kodak Tri-X film and 3 to 6 minutes on Ektachrome film.

RESULTS

I. Distribution of specific and nonspecific fluorescence in the stomach

Serotonin-fluorescent cells: The handdrum-shaped or plectrum-shaped, pale-yellow or yellow fluorescent cells were seen in the base of epithelium (Fig. 15, 17). These structures represented serotonin-containing enterochromaffin cells. Another kind of serotonin-fluorescent cell was mast cell located near the small artery (Fig. 6, 14, 17). The shape was round or polygonal and the fluorescence was bright orange-yellow or yellow, and brilliant.

Catecholamine-fluorescent nerve fibers: The green or green-yellow catecholamine fluorescence was seen around the small arteries and arterioles, along the layers of bundles of muscle fibers and in the myenteric plexuses. In all the region of stomach, the bright green catecholamine fluorescence was seen around the small arteries and arterioles. In a longitudinal section of blood vessel, a greenish fluorescent network was seen just like as an ivy around a tree (Fig. 11). Brilliantly fluorescent varicose structures stand in a line of the network. In a cross section, the fluorescent dots stand in a circle around the vessels. A few veins were surrounded by small number of fluorescent fibers but capillaries were devoid of any fluorescence (Fig. 20).

In all the region of stomach, the greenish fluorescent nerve fibers were seen in the outer longitudinal and inner circular muscle layers. Although distribution of the fibers varied according to region of the stomach, it was generally more dense in the inner layer than in the outer layer (Fig. 5, 11, 13, 19). Varicose structures were also seen. The fluorescent fibers run usually parallel to the muscle fibers and occasionally across them, and rarely run snaky.

Around the Auerbach's plexus located between outer and inner muscle layers, the greenish fluorescent nerve fibers were present. However, the cell bodies were non-fluorescent. Occasional fibers running in the muscle layer were found to reach the plexus. With a fluorescence microscope, the Meissner's plexus in the stomach was not identified. In contrast, the Auerbach's plexus, even when lost specific fluorescence,

was found clearly (Fig. 14). In the duodenum, the Meissner's plexus as well as the Auerbach's plexus were visualized definitely, irrespective of the intensity of fluorescence (Fig. 4).

Nonspecific autofluorescence: The surface of the flat epithelium in the nonglandular stomach showed pale-yellow autofluorescence (Fig. 11). Connective tissues also in part bore weak autofluorescence. However, it was not difficult to distinguish between autofluorescence and specific fluorescence. In cross-sectioned arteries, prominent autofluorescence was visible in the internal elastic membrane (Fig. 20). In the Auerbach's plexus and in the muscle layer proper, a large number of fine, orange or brown autofluorescent granules remained after disappearance of specific fluorescence by appropriate surgical procedures.

Mucosa: The stratified squamous epithelium of the nonglandular stomach showed the pale autofluorescence at the surface and showed the dark brown one in the deep (Fig. 11). Neither catecholamine-fluorescent nerve fibers nor serotonin-containing cells were seen. The glandular epithelia of the body and the antrum did not show any specific fluorescence. In the base of epithelium serotonin-containing enterochromaffin cells were identified (Fig. 13). Among and/or on these cells, serotonin-containing mast cells were present. Usually these two types of cells were easily discriminated each other. Glandular cells were not supplied by the fluorescent nerve fibers. In the lamina propria mucosa, serotonin-fluorescent mast cells were seen near the arterioles, and the green fluorescent nerve fibers were present in the wall of the blood vessels (Fig. 13). They both were smaller in number in the lamina muscularis mucosa.

Submucosa: Distribution of small arteries was rich in the submucosa, and they were surrounded by the catecholamine-fluorescent nerve fibers (Fig. 11). Occasional branches of the fluorescent fibers from the arteries run into the connective tissue fibers which separated the bundles of smooth muscle fibers. There was a large number of serotonin-containing mast cells in the submucosa, except in the antral portion where mast cell was rarely seen.

Muscularis: Small arteries accompanying the fluorescent nerve fibers, present in the connective tissue fibers which separated the layers of bundles of smooth muscle fibers, were seen to connect with those in the submucosa. The distribution of fluorescent nerve fibers in the inner muscle layer was more dense than in the outer muscle layer. Although no appreciable difference in density of the fluorescent fibers was found between nonglandular stomach and body, the density in the antrum was much smaller than in the above two portions (Fig. 11, 13, 15). The Auerbach's plexus was seen to receive the fluorescent nerve fibers.

Serosa: A large number of small arteries surrounded by the fluorescent nerve fibers was seen in the subserosal region. Serotonin-containing mast cell was small in number. In the serosa, neither fluorescent nerve fibers nor mast cell was visible.

II. Cervical vagotomy

Pieces of the nonglandular stomach, the body of stomach, the antral portion, the cardia, the duodenum and the esophagus were taken from rats which were killed 7 and 14 days after unilateral cervical vagotomy. In any pieces no detectable change

in intensity of catecholamine fluorescence was observed around small arteries, along layers of bundles of smooth muscle fibers and in the myenteric and submucosal nerve plexuses. The distribution and intensity of serotonin-fluorescent cells also did not show any appreciable change.

III. Abdominal vagotomy

Right- or left-side vagotomy was done in 5 rats each, 1 to 7 days prior to sacrifice. There was no significant change in distribution and intensity of catecholamine-fluorescent nerve fibers and serotonin-containing cells. Fluorescent materials were seen to accumulate just proximal to ligation of the cervical or abdominal vagus (Fig. 6).

In the case of bilaterally vagotomized rat, the specific fluorescence in the duodenum and in the stomach except cardia was affected nonsignificantly. The intensity of catecholamine fluorescence in the cardia was somewhat less than that in control animals (Fig. 9). The lower esophagus of operated rat showed a significant decrease in intensity of the fluorescence in vascular, muscular and plexus regions (Fig. 6). No significant change was observed in serotonin-containing cells.

IV. Splanchnicectomy

Right- or left-side splanchnicectomy was carried out in 5 rats each, 1 to 7 days before killing. Four rats were subjected to bilateral surgery. No appreciable change in catecholamine fluorescence was found in sections taken out from splanchnicectomized rats.

V. Celiac ganglionectomy

Seven rats were subjected to celiac ganglionectomy with a purification of connective tissue and nerve plexus surrounding the abdominal artery, 1 to 14 days prior to sacrifice. The catecholamine fluorescence in the lower esophagus was unaffected by the surgery. In the cerdia, the intensity of fluorescence was slightly less than that of control animals. In the remaining portions of stomach and the duodenum, there was a significant decrease in intensity of catecholamine fluorescence (Fig. 12, 14, 16, 17, 18). The intensity and number of serotonin-fluorescent cells were not significantly different from control preparations.

VI. Celiac ganglionectomy and bilateral abdominal vagotomy

Five rats were subjected to the surgery 1 to 14 days before killing. In any sections, the catecholamine-fluorescent nerve fibers were never seen around blood vessels, along the muscle fibers and in the myenteric and submucosal nerve plexuses of the upper gastrointestinal tract (Fig. 10). Only a few nerve fibers in the muscularis remained fluorescent. Under such conditions, no significant change occurred in the serotonin fluorescence.

DISCUSSION

In the stomach wall of rat, there were abundant catecholamine-fluorescent nerve fibers[31]. A large part of them was seen to surround blood vessels. NORBERG[42] has suggested that compared to the dense networks of fluorescent nerve terminals around the intramural ganglion cells and in the walls of the blood vessels, the muscle layers proper receive only a small amount of adrenergic innervation and furthermore, the innervation appears mostly to belong to small blood vessels. Very few varicose

Table 1. Noradrenaline-Fluorescence after Various Procedures

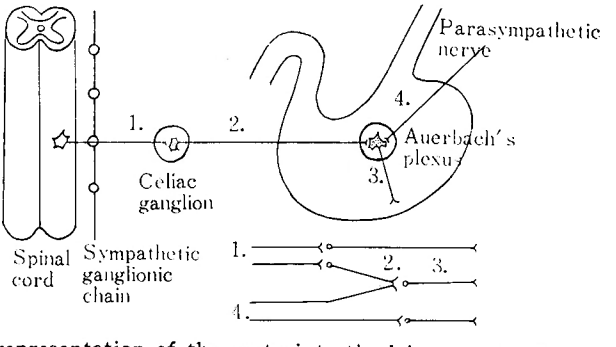
	Cervical Vagotomy	Abdominal Vagotomy uni-lateral	Abdominal Vagotomy bi-lateral	Splanch-nicectomy	Ganglio-nectomy	Bi-lateral Abdominal Vagotomy and Ganglio-nectomy
Oesophagus						
Blood vessel	+	+	−	+	+	−
Muscle layer	+	+	−	+	+	−
Auerbach's plexus	+	+	−	+	+	−
Cardiac region						
Blood vessels	+	+	±	+	±	−
Muscle layer	+	+	±	+	±	−
Auerbach's plexus	+	+	±	+	±	−
Body Non-glandural region						
Blood vessel	+	+	+	+	−	−
Muscle layer	+	+	+	+	−	−
Auerbach's plexus	+	+	+	+	−	−
Body Glandural region						
Blood vessel	+	+	+	+	−	−
Muscle layer	+	+	+	+	−	−
Auerbach's plexus	+	+	+	+	−	−
Antrum						
Blood vessel	+	+	+	+	−	−
Muscle layer	+	+	+	+	−	−
Auerbach's plexus	+	+	+	+	−	−
Duodenum						
Blood vessel	+	+	+	+	−	−
Muscle layer	+	+	+	+	−	−
Auerbach's plexus	+	+	+	+	−	−
Meissner's plexus	+	+	+	+	−	−

fibers observed in the mucosal layer were also suggested to belong to the blood vessels. CHRISTENSEN[5] has shown that there is a large number of adrenergic vasomotor fibers in the muscle layers of stomach. JACOBOWITZ[22] has also reported that there are few adrenergic fibers in the muscle layer proper of the gut and suggested that the fibers might be vasomotor in nature. On the other hand, MURYOBAYASHI et al.[37)39] described the distribution of catecholamine-fluorescent nerve fibers in the stomach of rat and pointed out their abundant presence along or within the circular and longitudinal muscle layers independently of the blood vessels. HOLLAND et al.[19] also observed the presence of adrenergic fibers, nonvasomotor in nature, in the muscle layers of the duodenum and large intestine of rat. The fluorescent nerve fibers in the submucosa clearly belonged to blood vessels[13)18)26)28)34)44)47]. In contrast, the fibers in the muscle layer proper run generally straight and nonsnaky. Experimental studies of injecting a plastic into blood vessels of the stomach[36] and of staining the myeline sheath of nerve fibers have shown that it is unlikely that most of arterioles run in parallel with the muscle fibers and they do not make any branching. Furthermore,

despite the abundant presence of the fluorescent nerve fibers, there was only a few blood vessels in the muscle layers. These findings suggest that the nerve fibers seen in the muscle layers do not belong to blood vessels but mostly supply the muscle layer proper.

In the lamina musclularis mucosa, the fluorescent nerve fibers were very small in number and located closely to blood vessels. In the lamina propria mucosa, the fluorescent nerve fibers were mainly found at the bottom, and there was no fiber at the surface epithelium and the treetop of villi. These observations indicate that arterioles in the villi are not supplied by the adrenergic nerve fibers and that the glandular secretion is not directly controlled by the nerve impulse. On the other hand, JACOBOWITZ[22] has reported that the fluorescent adrenergic nerve fibers are present in the epithelium of gastrointestinal tract of cat and monkey. The discrepancy might be due to a species difference.

The Auerbach's plexus of the stomach and duodenum was surrounded by the fluorescent nerve fibers. A similar finding was reported with the esophagus[40], small intestine[22)42] and large intestine[2)10]. It has been generally considerd that the preganglionic fibers of parasympathetic nerve is long and make plexuses in the wall of organ to which they distribute[4)27]. No synaptic contact has been considered to exist between the sympathetic fibers and the myenteric nerve plexuses[50]. NORBERG[42)43] and JACOBOWITZ[23] have suggested that the intestinal adrenergic nerves, apart from the vasomotor nerves, terminate mainly in the enteric plexuses, where they are in synaptic contact with the intramural ganglion cells. It was thus inferred that the sympathetic inhibition of intestinal motility is mediated indirectly by an effect on the postganglionic parasympathetic neurons. With a fluorescence microscope the Meissner's plexus in the duodenum was seen to be supplied by fluorescent nerve fibers. The plexus was unidentified in the stomach and esophagus[48]. NISHIMURA[40] also did not find fluorescent, submucosal plexus of Meissner in the esophagus of rabbit. Some reports demonstrated the presence of the plexus accompanying catecholamine-fluorescent nerve fibers in



Fig, 1. Schematic representation of the gastrointestinal innervation[43].
1: Preganglionic cholinergic fiber with its cell body in the spinal cord, terminating in the celiac ganglion. 2: Postganglionic adrenergic fiber with its cell body in the celiac ganglion, terminating in the Auerbach's plexus. 3: Postganglionic cholinergic fiber with its cell body in the Auerbach's plexus terminating in the gastrointestinal wall. The intramural ganglion cell (in the Auerbach's plexus) has a double innervation: One is parasympathetic preganglionic cholinergic nerve (4), the other (2) is sympathetic postganglionic adrenergic nerve.

the small and the large intestine[19)22)41)49).] It has been generally thought that the Meissner's plexus controls glandular secretion of gastrointestinal organs. Thus, absence from adrenergic innervation of the Meissner's plexus of the stomach might suggest that a mechanism of glandular secretion of stomach is different from that of duodenum.

CHRISTENSEN[5)] has described that the sympathetic nerve fibers are mixed in the vagus nerve which run near the sympathetic ganglion in the cervical region. MURYOBAYASHI et al[38)]. have demonstrated the presence of the fluorescent adrenergic nerve fibers in the cervical vagus and in its gastric branch of cat and dog. The present author also observed catecholamine fluorescence in the ligated abdominal vagus and its branches of rat. The denervation experiment of the vagus nerve at cervical and abdominal levels showed nonsignificant change of catecholamine fluorescence in the stomach except cardia region where the intensity and number of fluorescence decreased after bilateral abdominal vagotomy. The celiac ganglionectomy, in contrast, diminished the fluorescence in the stomach. The decrease was slight only in the cardia. The catecholamine fluorescence in the lower esophagus remained unchanged after celiac ganglionectomy but disappeared after abdominal vagotomy. The combination of abdominal vagotomy and celiac ganglionectomy resulted in a complete disappearance of fluorescence in both lower esophagus and stomach. NISHIMURA[40)] also has reported that cervical vagotomy decreases catecholamine fluorescence in the esophagus of rabbit. These observations indicate that the adrenergic nerve fibers present in the vagus do not distribute to the stomach except cardia region but mainly to the lower esophagus[32)]. The cardia region seems to be innervated by the adrenergic nerve fibers originating in the vagus nerve as well as from the celiac ganglion. The fact that the catecholamine fluorescence was unaffected at all by the cervical vagotomy indicates that the adrenergic nerve fibers distributing to the lower esophagus and the cardia make synapse lower than the cervical level.

DAHLSTRÖM[9)] demonstrated the accumulation of catecholamine-fluorescent materials in the splanchnic nerve of rat after ligation. The splanchnicectomy did not cause

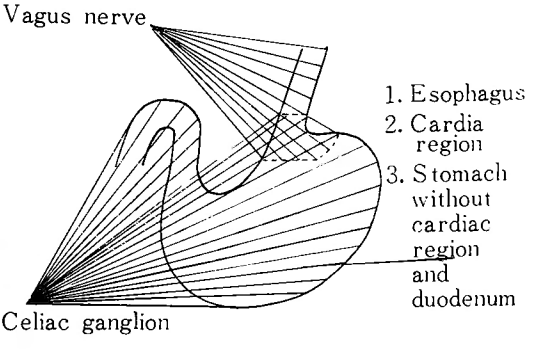


Fig. 2. Adrenergic innervation of the gastrointestine.
1. Esophagus is innervated with the adrenergic fibers running in the vagus nerve.
2. Cardia region is innervated with the both adrenergic fiber running in the vagus nerve and one from the celiac ganglion.
3. Stomach and duodenum are innervated with the adrenergic fiber from celiac ganglion.

any change in the catecholamine fluorescence of the stomach and duodenum[20]. However, the celiac ganglionectomy resulted in a disappearance of the fluorescent nerve fibers in both stomach and duodenum. These findings suggest that only a small part of, if any, adrenergic nerve fibers distributing to these organs passes through the celiac ganglion without making synapse.

SUMMARY

Using a fluorescence histochemical technique of FALCK and HILLARP, the mode of the adrenergic innervation to the stomach of rat was investingated. The following results were obtained.

1. In the stomach wall the adrenergic nerve fibers were seen around the blood vessels, along the layeres of bundles of muscle fibers, and in the myenteric plexuses. The fibers surrounding the blood vessels were found in arteries, arterioles and some veins in all the region of stomach. In the muscle layers, the adrenergic nerve fibers distributed directly to the smooth muscle fibers proper, independent of blood vessels. The Auerbach's plexuses of the esophagus, of the stomach, and of the duodenum were surrounded by adrenergic nerve fibers, but the Meissner's plexus, except in the duodenum, had no adrenergic nerve fiber.
2. The abdominal vagus nerves contained the adrenergic component in course. The fibers distributed to the esophagus and the cardia region of stomach but not to the remaining part of stomach and the duodenum. The cardia region of the stomach received adrenergic nerve fibers from the vagus nerve and from the sympathetic celiac ganglion. The remaining part of stomach and the duodenum received the fibers only from the celiac ganglion.

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Professor Dr. CHUJI KIMURA for his constant interest and guidance in this research. Thanks are also due to Former Professor Dr. KIRO SHIMAMOTO, Professor Dr. MOTOHATSU FUJIWARA and Dr. CHIKAKO TANAKA, Department of Pharmacology, Kyoto University, for their valuable suggestion and encouragement and to Dr. KENGO TSUNEKAWA for his kind advice and helpful assistance throughout the course of this work.

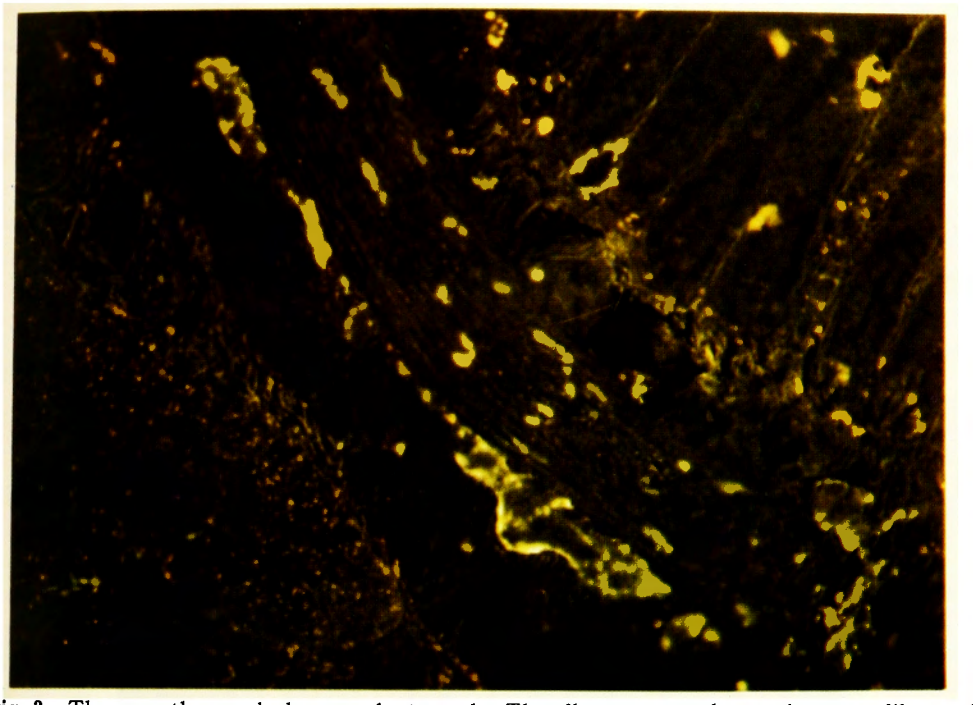


Fig. 3. The smooth muscle layers of stomach. The fluorescent adrenergic nerve fibers with varicose structures are observed along the muscle fibers without accompanying blood vessel. Around the blood vessel (→) the fibers are located on the outer border of muscle layer. Fluorescence Microphotograph ×160.

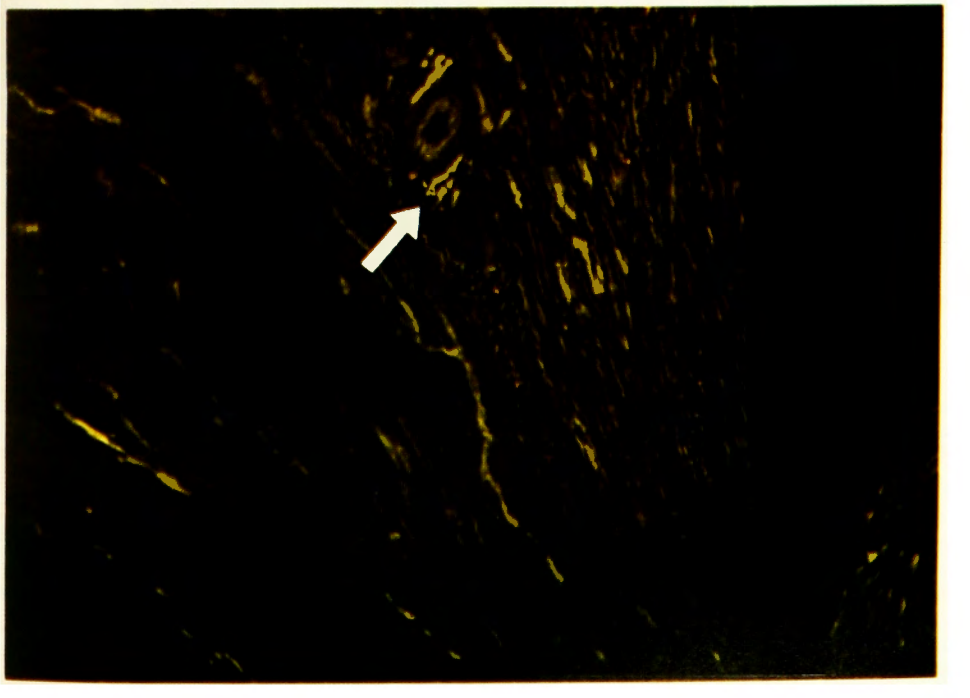


Fig. 4. Normal duodenum. Auerbach's plexuses are surrounded with adrenergic nerve fibers. In the sumbucosal region Meissner's plexus shows a similar structure. ×160.

Fig. 5

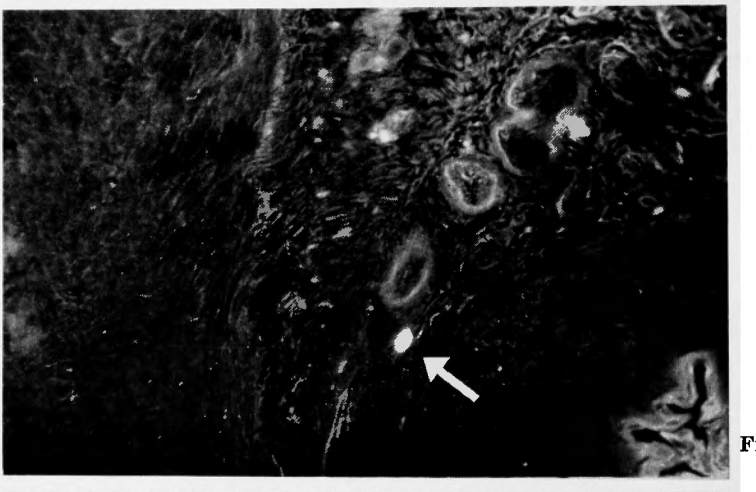


Fig. 6

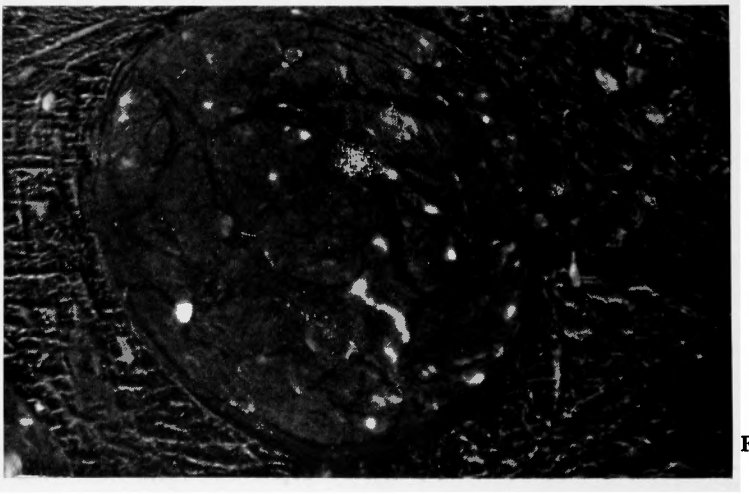


Fig. 7

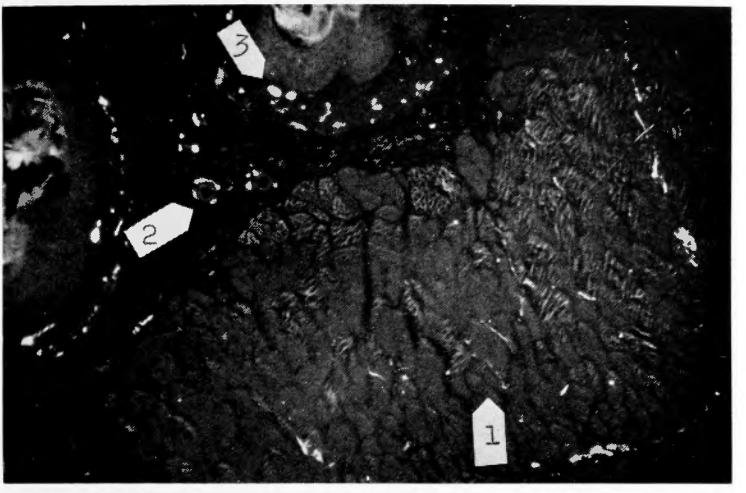


Fig. 8

Fig. 9

Fig. 10

Fig. 11

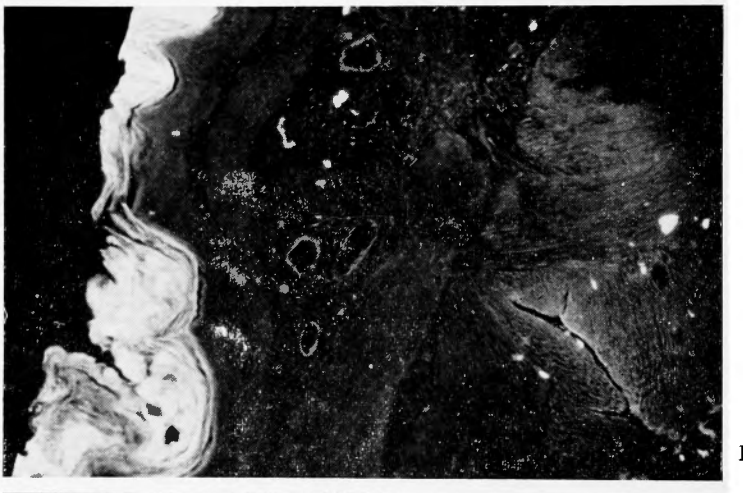


Fig. 12

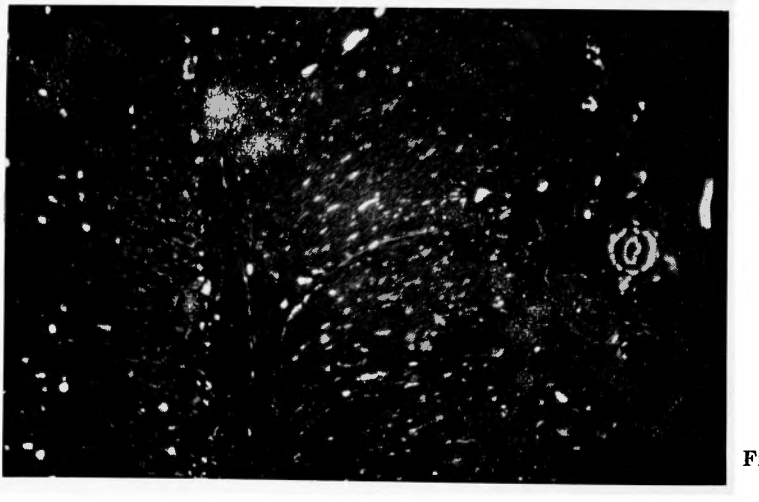


Fig. 13

Fig. 14

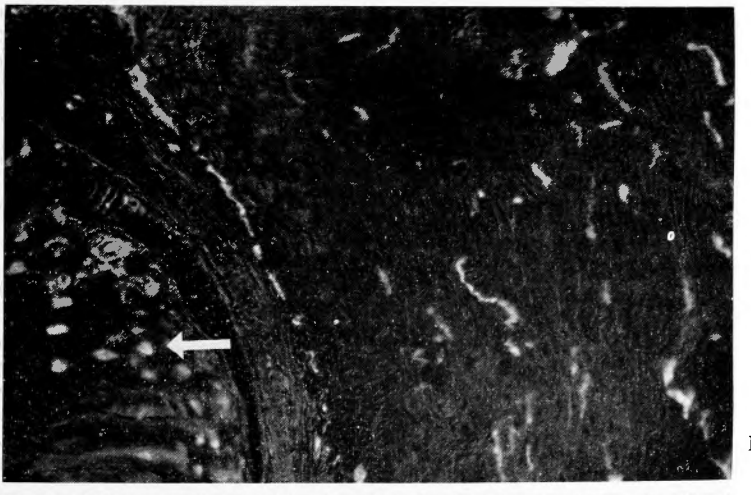


Fig. 15

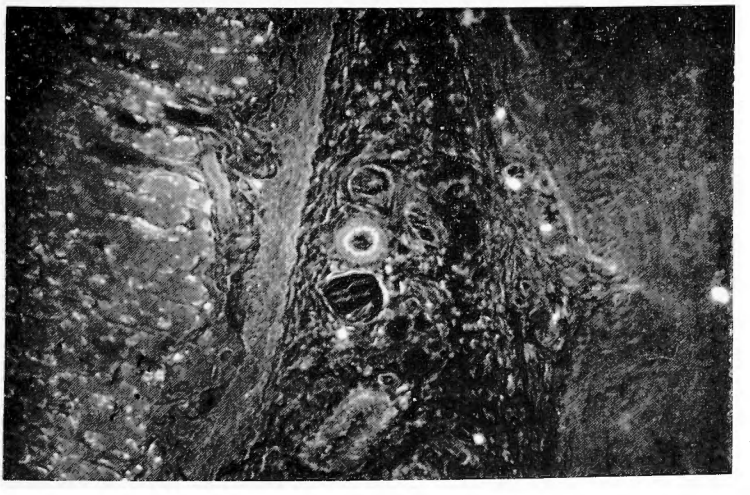


Fig. 16

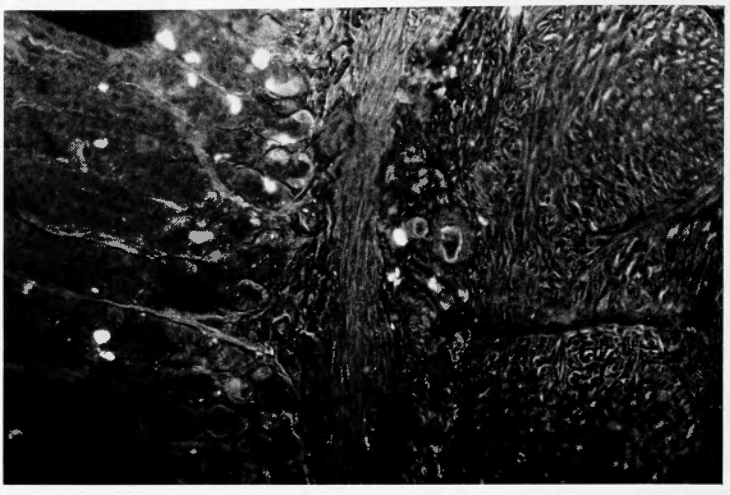


Fig. 17

Fig. 18

Fig. 19

Fig. 20

附　図　説　明

Fig. 5. Normal esophagus. Adrenergic nerve fibers are observed around the blood vessel (1), along the muscle fibers (2), and in the Auerbach's plexus. The surface of mucous membrane shows non-specific auto-fluorescence (3). ×63.

Fig. 6. Denervated esophagus. There is no adrenergic nerve fiber (5 days after bilateral abdominal vagotomy). The remaining fluorescence (→) represents serototonin of mast cells. ×160.

Fig. 7. Vagus nerve 24 hours after ligation. Accumulation of catecholamine fluorescent materials. This indicates that the vagus nerve contains adrenergic component. ×160.

Fig. 8. Normal cardia region of stomach. (1) Adrenergic nerve fibers in muscle layer, (2) adrenergic nerve fibers around blood vessels, (3) serotonin of mast cells. ×63.

Fig. 9. Cardiac region of stomach 5 days after bilateral vagotomy. A few nerve fibers remain fluorescent. ×160.

Fig. 10. Cardiac region of stomach 5 days after the combined procedures of the bilateral abdominal vagotomy and the celiac ganglionectomy. Complete disappearance of adrenergic nerve fibers. ×160.

Fig. 11. Non-glandural region of stomach. The mucous epithelium shows a non-specific auto-fluorescence. In the submucous region, many blood vessels with adrenergic nerve fibers are observed (1). In the muscle layer, there are abundant adrenergic nerve fibers (2). Auerbach's plexus are surrounded by adrenergic nerve fibers. Meissner's plexus is not seen. ×63.

Fig. 12. Denervated nonglandural region of stomach, 7 days after the celiac ganglionectomy. Adrenergic nerve fibers completely disappear. Remaining fluorescence represents serotonin of mast cells. ×63.

Fig. 13. The normal body of stomach. Abundant adrenergic fibers are observed in the smooth muscle layer. ×63.

Fig. 14. The denervated body of stomach (7 days after celiac ganglionectomy). Complete disappearance of noradrenaline fluorescence and remaining of serotonin fluorescence. ×63.

Fig. 15. Normal antrum. In the smooth muscle layer many adrenergic fibers are observed. Among the glandural cells, there is a large number of serotonin fluorescence (→). ×160.

Fig. 16. Denervated antrum. 7 days after celiac ganglionectomy. No noradrenaline fluorescence is seen. Many mast cells show serotonin fluorescence. ×160.

Fig. 17. Denervated antrum. Serotonin fluorescence of mast cells. In the glandural region, the distinction between the two types of fluorescence is difficult. ×160.

Fig. 18. Denervated duodenum. 7 days after the celiac ganglionectomy. The noradrenaline fluorescence along the Auerbach's plexus and in the muscle layers completly disappears. ×160.

Fig. 19. The adrenergic fiber with varicose structure in the smooth muscle layer of cardiac region. ×160.

Fig. 20. Adrenergic fibers around the blood vessels. Artery has many fluorescent fibers on the outer border of muscle layer. Vein has generally no adrenergic fiber. But occasional vein has a few fibers (→). ×160.

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和文抄録

組織化学的蛍光法によるラットの胃のアドレナリン作動性神経支配に関する研究

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胃の機能は複雑である．それは食物の受納であり，消化液の分泌であり，両者を混合して行う消化であり，更にこれを十二指腸へ送り込む事である．これらの複雑な機能が，精致な液性或は神経性調節機構の下に営まれている事に疑問はない．1899年，Bayliss と Starling が，胃の運動は迷走神経と内臓神経の拮抗的な支配下にある事を明かにして以来，胃の機能の調節機構に関する種々の解剖学的或は生理学的追求が行われ，数多の知見をもたらしてきた．

胃は迷走神経及び内臓神経を介して神経繊維を供給されている．今日迄，胃の神経分布の研究は，肉眼的，或は神経繊維の変性又は好銀繊維の染色といった方法で行われてきた．しかしこれらの形態学的方法では，内臓神経と交感神経，或は迷走神経と副交感神経を区別する事さえ不可能であり，事実これらはしばしば混同されていた．この点，Falck 等によって開発されたカテコールアミンの組織化学的検出法は，交感神経節後繊維のみを特異的に検出でき，しかも細胞レベルでの追究が可能である為，交感神経の研究には傑出した手法である．

既にこの方法を用いた研究は各地で行われ，種々の知見をもたらしており，消化管についても，人及び多くの動物についての観察の結果が報告されている．しかしながら，胃の交感神経支配に関して，その支配神経の起源並びに径路を追究したものは，未だこれを見ない．

著者はこの組織化学的螢光法を用いて，ラットの胃壁に於けるアドレナリン作動性神経繊維の分布の様相を観察すると共に，その神経繊維の起源と胃に至る径路を，種々の外科的手法に依って追究した．

胃壁に分布するアドレナリン作動性神経繊維は3種に分類される．その1は血管周囲にあって，これと共に走行分布するものであり，その2は平滑筋層にあって，血管に伴走する事なく，直接筋繊維に分布するものであり，その3は神経叢中に見られるものである．

血管壁にアドレナリン作動性神経繊維のある事は，既に各種の動物のあらゆる組織に於て観察され報告されている事であり，胃も亦，その同じ形態を示す器管の1つである．一方平滑筋層内に見られる繊維については，従来一般に，筋層内血管壁のものと考えられていた．しかしながら，粘膜下或は漿膜下及び他の臓器に於ける血管とアドレナリン作動性神経繊維との関係を参考にしてこれを見れば，この考え方は誤りである事が判る．即ち筋層内に於ては，神経繊維が非常に豊富であるにも拘らず，殆んど血管の断面を見ない．又若しこれを毛細血管壁に分布する神経繊維と考えるなら，身体各所で未だ毛細血管にアドレナリン作動性神経繊維を見られた事がないという事実に反する．更に此処に於ける神経繊維の走行が筋繊維の走行に沿っている事は，血管の走行と著しく異なる点である．従って筋層内のアドレナリン作動性神経繊維は，血管壁に分布するものではなく，全く独立して，直接筋繊維に分布するものと考えざるをえない．次にアウエルバッハ神経叢にアドレナリン作動性神経繊維を見る事は，今日迄の自律神経学の定説に1つの訂正を加える所見である．即ち，従来，アドレナリン作動性神経繊維は，交感神経節後繊維としてのみ存在するとされていたのであるが，ここに於て，アドレナリン作動性神経繊維が，或る意味で節前繊維として機能している事が示されるのである．一方マイスネル神経叢に関しては，十二指腸に於てはアウエルバッハ神経叢と同じくアドレナリン作動性神経繊維を見るにも拘らず，胃に於ては全くこれを見ない．これは，胃と十二指腸の分泌機構に，神経支配上の差異のある事を示す所見である．

従来，迷走神経が頸部に於て交感神経幹と密接な関係にある点から，迷走神経中に交感神経繊維が含まれるのではないかという考えがあった．無量林は迷走神経を結紮し，その結紮部にアドレナリン様物質の貯留する事を観察し，この考えの正しい事を証明した．一方，大内臓神経を結紮すると，その結紮部に同じくアドレナリン様物質の貯留する事が報告されている．著者は頸部及び腹部迷走神経切断，内臓神経切断，腹腔神経叢剔清，及び迷走神経切断と腹腔神経叢剔清を併用する事に依り，迷走神経及び内臓神経中のアドレナリン作動性神経繊維の分布部位を追求した．その結果，迷走神経中のアドレナリン作動性神経繊維は，下部食道及び胃の噴門部に分布する事を知った．一方胃に分布するアドレナリン作動性神経繊維は殆どが腹腔神経節起源のものであり，内臓神経中に在って，この神経節を通過して胃に至る繊維は極めて少ない事を知った．そして又，胃の噴門部は，そこに分布するアドレナリン作動性神経繊維を，迷走神経と腹腔神経叢の両方から得ている事が判った．